Delayed Type Hypersensitivity: Current Theories with an Historic Perspective

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●Abstract

Although the delayed type hypersensitivity (DTH) reaction was discovered over 100 years ago, the exact nature of the reaction has been the subject of contentious debate over the years. The reaction was discovered in 1882 by Robert Koch, but it wasn't until the 1940s that Landsteiner and Chase proved that the reaction was mediated by the cellular and not the humoral arm of the immune system. The first DTH reaction described used only the tuberculin antigen (tuberculin reaction), but the definition was later expanded to include cell mediated reactions to other bacterial and viral antigens, responses to pure protein with adjuvant or haptens, and host responses to allograft. The DTH skin test is used to test if prior exposure to an antigen has occurred. When small quantities of antigen are injected dermally, a hallmark response is elicited which includes induration, swelling and monocytic infiltration into the site of the lesion within 24 to 72 hours. This reaction has been shown to be absolutely dependent on the presence of memory T cells. Both the CD4+ and CD8+ fractions of cells have been shown to modulate a response. Contemporary debate regarding the reaction is focused on the role of the Th1 and Th2 cells originally discovered by Mosmann. It has been postulated that the Th1 cell is the "inducer" of a DTH response since it secretes interferon gamma (IFN γ), a potent stimulator of macrophages, while the Th2 cell is either not involved or acting as a downregulator of the cell mediated immune response. Despite the early experimental success of this theory, experiments have shown that Th2 cells may be involved in certain types of proinflammatory cell mediated immunity. This review focuses on the nature of the different forms of DTH that can be elicited and the different experimental evidence

that has led to the current theories regarding DTH and its role in cell mediated immunity.

●Introduction

The delayed type hypersensitivity was the first experimental evidence of transferable immunity carried only by the immune cells. The original debate about the role of cell mediated and humoral immunity began in the 19th century between the French "cellularists," led by Elie Metchnikof and the German "humoralists." The humoralists believed that immunity was due to serum factors (antibodies and complement) which directly destroyed bacteria. The cellularists believed that phagocytes were the basis for immunity.[1] By the early 1900's the "humoralists" had ostensibly won the debate, with biochemical characterization of antibodies and complement. However, by the 1940's experiments confirmed that both theories were essentially correct. Immune function is not only chemical (antibodies, complement) but also cellular (T cells, B cells and macrophages). Robert Koch, the discoverer of the tubercule bacillus was the first to demonstrate a delayed type hypersensitivity reaction in 1882.[2] Koch attempted to use his killed tuberculin preparation as a prophylactic and therapeutic vaccine. Unfortunately, the antigen did not confer protection to naive patients, and when injected intravenously in infected patients, caused reactivation of the disease and in some cases death. Nevertheless, when the antigen was injected intradermally, the delayed inflammatory response (tuberculin reaction) could indicate whether or not an asymptomatic person had been exposed to Mycobacterium tuberculosis. It was not until 1942 that Landsteiner and Chase demonstrated that the DTH reaction could be transferred by a "cell only" fraction.[3] The basis for the experiment was fairly direct. Cells from guinea pigs, which had been immunized with Mycobacterium tuberculosis or hapten, were transferred into naive guinea pigs. Later, when antigen or hapten was injected into these guinea pigs, they elicited an immune

recall response that was not present in the naive controls.[4] This did not happen when the serum fraction was transferred. Coombs and Gell classified delayed type hypersensitivity as Type IV.[5] The three other classifications of hypersinsitivity are Type I (immediate/IgE-related) in which the cutaneous skin test reaction peaks at 2 hours, Type II (antibody and complement related cytotoxicity) and type III (antigen-antibody complex mediated).

DTH responses have been well characterized. The reaction is antigen specific and causes erythema and induration at the site of antigen injection in immunized animals or humans. Systemic injection of antigen results in fever, synthesis of acute phase proteins and in some instances death. The nature of this antigen can be varied. Mycobacteria, protein, hapten, and even grafted tissue are all capable of inducing delayed type hypersensitivity reactions. The histology of DTH can be different for different species, but the general characteristics are an influx of immune cells at the site of injection, either macrophages and basophils in humans and mice or neutrophils in guinea pigs, and induration which becomes apparent within 24-72 hours. Even though they make up only a small percentage (10-20%) of the total inflammatory infiltrate at 48 hours, T cells (either CD4+ or CD8+ depending on the antigen) are required to initiate the reaction.[6,7]

●The subclasses of delayed type hypersensitivity

Tuberculin Reaction

The classic form of DTH is induced by injecting an antigen preparation of Mycobacterium tuberculosis intradermally. If the host has been previously exposed to the bacterium, swelling and induration will result. Waksman provides a summary of the DTH[8]: "Approximately 4 hours after injection of antigen,

neutrophils rapidly accumulate around the post-capillary venules at the injection site. The neutrophil infiltrate rapidly subsides and by about 12 hours the injection site becomes infiltrated with T cells and blood monocytes and some basophils, also organized in perivenular distribution. The endothelial cells lining these venules swell, show increased biosynthetic organelles and become leaky to plasma macromolecules. Fibrinogen escapes from the blood vessels into the surrounding tissues, where it is converted into fibrin. The deposition of fibrin and to a lesser extent accumulation of T cells and monocytes within the extravascular tissue space around the injection site cause the tissue to swell and become indurated. Induration, the hallmark of DTH, is usually detectable by about 18 hours and maximal by 24h in mice, 48h in guinea pigs, and 48-72 hours in humans. The inflammation then subsides. Of note, is the fact that guinea pigs have a significantly different response to the tuberculin reaction. Instead of monocytes, the primary infiltrate is neutrophils." In all species, this reaction is mediated by a mix of CD4+ and CD8+ T cells. This may partly be a result of the fact that Mycobacteria have both an intracellular and extracellular replication phase.[9]

Jones-Mote Hypersensitivity: Protein-Adjuvant Reactions

Closely related to the tuberculin reaction, is the host response to pure protein mixed with an adjuvant. This form of DTH was discovered in 1929 by Louis Dienes. He demonstrated that when ovalbumin, an egg white protein that is normally not immunogenic, is injected into a tuberculosis tubercule, the patient would become sensitized to the protein.[10] Later with the introduction of Freund's adjuvant, the reaction could be mimicked by mixing the protein with killed mycobacterium in oil.[11] When it was discovered that any pure protein mixed with adjuvant could induce an immune response, the DTH reaction was termed the Jones-Mote reaction since it was fundamentally different from the tuberculin reaction in one remarkable aspect.[12,13] If the protein was injected intravenously before the immunization with hapten-

conjugated protein or protein in adjuvant, then sensitivity was abolished. This was the first demonstration of immunological tolerance, and it was not inducible with the tuberculin antigen itself.[14] Jones-Mote hypersensitivity exclusively refers to DTH induced by injection of protein in adjuvant or protein conjugated to haptens.

Jones-Mote hypersensitivity is identical in time-course to the tuberculin response but a greater number of basophils are found. In some texts this reaction is termed cutaneous basophil hypersensitivity. The response is mediated by the CD4+ T cell.[15] When this reaction becomes chronic, the inflammation is characterized as a granulomatous reaction. The primary T cell involved in the granuloma formation can be either a CD4+ or CD8+ T cell, depending on the nature of the antigen. CD4+ cells regulate the response to protein and extracellular pathogens. But when the infection is viral or intracellular, CD8+ T cells are predominantly involved, and can also adoptively transfer the DTH reaction.[16] In the case of murine viral lymphocytic choriomeningitis, infection is characterized by inflammation of the meninges and choroid plexus. In mice that are immunosuppresed or tolerized, no disease develops even though large levels of virus can be found.[17] With the transfer of sensitized CD8+ cells the inflammation again occurs. For the intracellular parasite Leishmania major, experiments in mice have shown that both CD4+ and CD8+ cells are involved in the disease.[16, 18]

Jones-Mote Hypersensitivity: Contact Hypersensitivity

Contact hypersensitivity (CHS) is another form of T cell mediated immunity that is characterized as DTH and is closely related to the protein in adjuvant reaction. It was originally described by Bennacerraf and Gell.[5] It can be induced experimentally by painting hapten on the skin and mimics the reactions seen to poison ivy and to various drugs and industrial or household chemicals. It can also be induced by conjugating the immunizing

protein to a hapten carrier and then injecting the complex.[8] In human skin the contact hypersensitivity lesion from hapten-painting is eczematous or vesicular in appearance. Similar to the Jones-Mote protein-adjuvant reaction, it has a marked basophilic infiltrate that invades the epidermis, but evolves more slowly, reaching a peak in three to six days.[8]

The mechanisms of CHS are now becoming understood. There are three critical events that must occur in generating a reaction, sensitization, trafficking and elicitation. In the sensitization phase, a naïve subject is exposed to hapten, usually through the skin but sometimes though inhalation or ingestion. Usually no symptoms of exposure are evident. The hapten binds covalently to any cell-associated protein or extracellular protein. The chemically modified proteins can then be presented by antigen presenting cells (APC) to T cells which recognize the modified protein as foreign. The molecular targets of most environmental haptens are not well described, but experimental haptens such as trinitrobenzene sulfonic acid and picryl chloride primarily bind lysine and other nucleophilic side chains via an amide bond.[19] The bulky benzene ring and the modified protein can then be recognized by antibodies as well as T cells in the context of MHC class I and MHC class II. Once the APC has pinocytosed or phagocytosed the protein, it will traffic back into the draining lymph node where it will present the antigen to reactive T cells and expand the clonal population.[20] These memory T cell clones that are expanded in the lymph node then can generate the elicitation phase of the response.

In the elicitation phase, local APCs present the hapten-protein to transiting memory T cells. The T cells then recruit more inflammatory cells to the antigen deposition site. In skin sensitization, the local Langerhans' cells have been shown to play an important role by presenting modified-self antigen in context of

MHC class II.[21,22] However, it has also been demonstrated that CHS reactions can occur in a MHC class I restricted manner.[23] One possible mechanism that can explain these results is that keratinocytes can also act as APCs by presenting hapten-modified self proteins in the context of MHC class I molecules.[24] Another group has shown that Langerhans' cells present hapten-peptide in both the MHC class I and MHC class II molecules.[25] Although no consensus exists on the exact mechanism, it is clear that both MHC systems appear to be involved in the reaction. There is experimental evidence that CD4+ T cells regulate the magnitude and duration of the CD8+ T cell response.[26,27] In fact, some of the current evidence suggests that the CD8+cells may play a more important role in CHS since AIDS patients that are refractory for the tuberculin reaction still retain contact hypersensitivity reactions,[28,29] as do experimental MHC class II deficient animals.[23,25]

The molecular mechanisms involved in CHS are also becoming clearer. The antigen presenting cell whether it is a Langerhans' cell or another form of APC must not only recognize the T cell receptor and CD8+ or CD4+ molecule but also co-stimulatory molecules. The B7/CD28 ligand pair has been shown to be important in regulating the T cell response to contact sensitizers. Suppression of CHS can also occur. One important environmental factor that suppresses CHS in both elicitation and sensitization phases is local UV irradiation. Numerous mechanisms for this suppression have been proposed, including depletion of Langerhans' cells, aberrant regulation of cytokine secretion by both APCs and keratinocytes and down-regulation of co-stimulatory molecules on APCs.[30-32] An obvious effect of UV irradiation is the depletion of Langerhans' cells.[33] Experimental evidence indicates that the cell death is due to apoptosis or programmed cell death, presumably from extensive DNA modification by the UV light.[34] Langerhans' cells that do survive have unusual expression of the co-stimulatory molecule CD86 which

has been shown to deliver a negative regulatory signal to T cells.[30] UV mediated tolerance may also be relevant in the generation of melanoma since Langerhans' cells provide immunosurveilance against neoplasms.[35]

Graft versus Host Disease

Additionally, graft versus host disease (GVHD) is a result of cellular immunity and is an example of a DTH response. A rejected allograft has a similar histological appearance to a tuberculin reaction and rejection is mediated by T cells with a clear role for the NK cell. Peter Medawar first discovered that allograft host rejection is a form of cell mediated immunity.[36] He was impressed by the marked monocytic infiltrate in a rejected organ which resembled the tuberculin reaction and although originally he characterized the response as a Type II, Arthus reaction, subsequent experiments confirmed that it was actually a Type IV, DTH response. Experiments of Brent, Brown and Medawar showed that injection of a cellular extract of alloreactive cells, not serum, could induce the classic DTH response if injected subcutaneously into a guinea pig which had rejected a transplant, but not in a naive animal.[37] The lesions resembled those of the acute tuberculin reaction, but there was also a late phase proliferation of large, pale, epitheloid histocytes. Also similar to the graft vs. host disease form of cell mediated immunity are some autoimmune diseases, Hashimoto's thyroiditis, Sjogren's disease, adrenalitis, polymyositis, and pernicious anemia. [8] The pathological picture is one of mononuclear cell infiltration and tissue destruction. Notably, the CD8+ T cell is the primary T cell inducing the lesions, although a minor role for CD4+ cells has been described.[8,38]

●Molecular Mechanisms of DTH

Cell mediated immunity as measured by DTH is described classically as fulfilling four criteria: 1) A requirement for T cells as evidenced by T cell transfer

techniques, 2) T cells are observed in the lesion itself, 3) no sensitization is possible in individuals with thymic aplasia (DiGeorge syndrome) or in experimental thymus-deprived animals, 4) existing sensitization is ablated by treatment with anti-lymphocyte serum.[8] The sequence of events involves numerous cell types and can vary in time course and histology. However, a model for the reaction is summarized by Abbas[39]: "Upon injection of the antigen, Langerhan's cells process the antigen and present it to local memory T cells, whether they are CD4+ or CD8+. These T cells in concert with activated Langerhan's cells secrete numerous cytokines that cause the early hallmarks of inflammation. Within 2 hours neutrophils begin to infiltrate the injection site. Currently, it is unknown if the T cells directly attract neutrophils and monocytes or if the vascular endothelium is responsible for recruitment of leukocytes, but the latter hypothesis seems more likely. During the early stages of inflammation, leukocytes migrate exclusively through the post capillary venules and do not follow an obvious gradient of cytokine. Although cytokines such as members of the chemokine family that are secreted by T cells and macrophages are chemoattractant for numerous immune cells, it is unlikely these cytokines are directly regulating the influx of cells from the vasculature. Instead, it is probable that the venular endothelium is recruiting the cells to the local site. The endothelial cells secrete vasodilators such as prostacyclin. T cells may be involved in inducing this response by secreting TNFα, which has been shown to increase the expression of prostacyclin producing enzymes. The vasodilatation caused by the prostacyclin optimizes delivery of immune cells to the site of challenge. The endothelial cells undergo changes as a result of TNFα and IFNγ acting in concert. The endothelial cells remodel the basement membrane and allow the extravasation of plasma macromolecules, especially fibrinogen. The increase in fluid volume slows the blood flow and allows the lymphocytes to attach more readily to the endothelium. Additionally, endothelial cells are also capable of secreting chemokines which attract various types of cells

into the site. Both IL-8 and MCP-1, both members of the chemokine family, have been shown to be produced by endothelial cells and are capable of increasing the mobility of adherent leukocytes from peripheral blood. The activated endothelium synthesizes numerous adhesion molecules such as ELAM-1, VCAM - 1 and ICAM-1. ELAM-1 selectively binds neutrophils. VCAM-1 binds VLA-4 present on lymphocytes and macrophages which also acts as a homing receptor for mucosal integrins. ICAM-1 binds LFA-1 on leukocytes. Patients with leukocyte adhesion deficiency (loss of LFA-1, Mac-1 and p150,95 proteins) have recurrent bacterial and fungal infections since polymorphonuclear cells cannot accumulate at sites of infection and they do not generate strong DTH skin test responses." Following this pattern, ICAM-1, P selectin, E selectin, and CLA1 have all been shown to be upregulated in local contact hypersensivity.[40-43]

Two types of T helper cells have been described, the Th1 and Th2 cell. The Th1 cell secretes interferon gamma, which activates macrophages and induces a cell mediated immune response.[44] Th2 cells secrete cytokines such as IL-4, IL-5 and IL-6, which activate B cells and induce humoral immunity or toleragenicity. The two types of responses appear to be mutually exclusive. Induction of the Th1 or Th2 phenotype is due to the antigen presenting cells secreting IL-12 which induces Th1 cells or secreting IL-10 which induces Th2 cells. Mosmann and others originally proposed that the Th1 cell that secretes interferon gamma is the only T cell capable of inducing a DTH reaction.[44] Th2 cells secreting IL-4, IL-5, and IL-6 were not believed to be involved in the DTH reaction, but current studies indicate that this may not be the case. Although Mosmann demonstrated that none of their own Th2 clones could induce a DTH reaction, other groups have produced Th2 clones which can.[45] Additionally, not all of the Mosmann's Th1 clones that secreted IFNγ could induce a DTH.[46] Xu reported in CHS that the type of hapten defined whether

the B7/CK28 signal is either inflammatory with Th1 cells being predominant or toleragenic with Th2 cells that generally express CD30, a memory lymphycyte marker. [47,48] However, others have described contact hypersensitivity reactions where both Th1 and Th2 cells have been detected.[49-51] Therefore the Th1 versus Th2 dichotomy may not always exist for the induction of delayed type hypersensitivity. The kinetics of the DTH responses are slightly different, with Th2 DTH lasting only 48 hours as opposed to 72 hours for Th1 DTH, but this is a relatively minor difference.[45]

Once cells have been recruited into the site, the cause and effect relationship becomes less clear. At least 15 different cytokines and inflammatory mediators have been described to be present at a local site of inflammation depending on the model used. These include IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, G-CSF, GM-CSF, PGE2, MCP-1, TNF α, TGF β, IFN α, IFN β, and IFN γ.[52, 53] However, of all of these cytokines, the most interest has been on the so-called Th1 and Th2 cytokines as well as TNFα in inducing or abrogating a DTH response.[54] It is clear that IFNγ can mediate a DTH response. It is found at high levels at the site of antigen injection in the case of fungal and bacterial antigens.[45,55,56] Direct injection of IFNγ induces an inflammatory infiltrate similar to that of injection with protein-adjuvant although it lasts for a shorter duration. [57] TNFα is also capable of inducing a local DTH response in humans and is more effective than even IFNγ. [54] Langerhans' cell derived IL-1β has been shown to be crucial in inducing a CHS and may act by inducing subsequent TNFα production.[58]

The late phase (24-72 hours post injection) of the Type IV DTH has not been closely studied. It is unclear what mechanisms allow lesion suppression or healing. Presumably the decrease in antigen concentration is partially responsible, though other factors are certainly involved. Some evidence points to a possible role of the

Th2 cells. Both IL-4 and IL-10 when injected systemically can decrease the magnitude of the skin test response once it has begun in a Leishmaniasis model, with IL-10 being the most potent.[18] When similar experiments were performed with contact sensitivity DTH, IL-10 was also able to reduce swelling.[59] When IL-10 was administered during the inductive phase of immunization in both the Leishmania model and contact hypersensitivity model no effect was observed. However, in one study, IL-10 downregulation was observed in the sensitization phase. If the hapten was conjugated to red blood cells then injected into the mice to sensitize as opposed to directly skin painting the animals, then IL-10 could downregulate the response at both the sensitization and elicitation phase of the reaction.[59] This indicates that the hapten-conjugate induced form of DTH is somehow different in its elicitation phase than direct contact sensitivity. Suppression of contact hypersensitivity by UV irradiation induces secretion of APC-derived prostaglandin E2, an immunosupressive steroid, keratinocyte-derived IL-10, and TNFα.[31,60,61] Other studies have shown that the epidermal CD8+ cell and its response to IL-12 may also play a role in UV-mediated tolerance.[59,62] Administration of IL12 blocks the induction of UV irradiation-induced tolerance and may act by downregulating suppressor CD8+ T-cells. IL-6 will decrease the swelling if injected 24 hours after the initiation of the response in the local site in an adjuvant arthritis model.[63] Systemically administered TGFβ in liposomes is also able to diminish the response but not when given as soluble protein.[64] If TNFα is administered during antigen sensitization in the Jones-Mote DTH, swelling is decreased by about 75%.[65] In the tuberculin reaction, if TNFα is injected into the antigen challenge site then swelling can be either increased or decreased depending on the original sensitizing antigen dose.[54] The cumulative data indicate a complex process of initiation and regulation during DTH, and although numerous cytokines participate, no one cytokine is able to ablate DTH, presumably because redundant systems are operating

during the inflammation.

Recently with the advent of gene "knockout" technology, experiments directly testing the role of these cytokines in vivo have been performed. Interestingly, the mice deficient in IFNγ, its receptor or TNFα receptor are all able to mount a DTH response.[66-68] In fact, the reaction is greater as measured by footpad swelling in knockouts than it is in wild type littermates. These results are the opposite of that predicted by the original Th1/Th2 theory of cellular immunity. Additionally, Coffman has demonstrated that direct injection of anti-IFNγ or anti-TNFα, either alone or in combination, cannot abrogate a DTH response in a Leishmaniasis model that has been used as the archetypal Th1 model.[18] In another model where the DTH reaction is caused by transfer of Th1 clones, Mosmann showed that anti-gamma interferon treatment only reduced the footpad swelling by one half. [69] Nevertheless, it is clear that IFNγ is intimately associated with the local response even though it may not be the initiator of DTH. IFNγ and TNFα can both be detected in situ and ex vivo in DTH reactions.[53,70,71] In human skin biopsies of tuberculin reactions, mRNA from different cytokines has been quantitated.[72] In 1000f the patients (n=10), IFNγ and IL-2 mRNA was detected. 600f the patients had no detectable Th2 cytokines but the other 40 0id show detectable levels of IL-4, IL-5 and GM-CSF. In patients with tuberculosis and positive tuberculin reactions, serum IgE which reacts against the mycobacterial antigen was found, and PBMCs stimulated with antigen produced IL-4.[73, 74)] If IL-4 were not involved in the tuberculin response, then IgE should not have been found since IL-4 is the IgE switch factor.[75] Therefore, DTH in humans is not required to be an exclusively Th1 response. Mosmann has modified the Th1/Th2 theory to accommodate these data. Current Th1/Th2 theory espouses that IL-4 is critical for generating a systemic DTH response. This is consistent with the fact that IL-4 deficient mice cannot mount a contact hypersensitivity response.[76] One group has recommended the designation DTH1 and DTH2 to

distinguish between pro-inflammatory cell mediated immunity elicited by either of the respective clones.[77] Another group found that depending on the dose of mycobacterial antigen or whether the mycobacterium was emulsified in Freund's adjuvant changed whether a Th1 or Th2 DTH was observed.[38] High doses of antigen or antigen in adjuvant led to a Th2 response which was non-protective, whereas low doses led to a Th1 response which was protective. Both regimes led to a positive DTH reaction as measured by footpad swelling.

Finally, it is necessary to view DTH not as an individual phenomenon but rather a group of related responses to antigen. These include the tuberculin reaction, Jones-Mote reaction, contact hypersensitivity and graft vs. host disease. These reactions can be further divided into CD4+ and CD8+ compartments and then subdivided on the basis of the T cells' cytokine secretion pattern. It is clear that cell mediated immunity must be highly adaptable and therefore variable. The phagocyte (monocyte/macrophage) is involved in all of these responses, sometimes as a host for the pathogen, but more often as an effector cell. The exact mechanism by which the macrophage is activated is still being debated but it is clear that a T cell is required to initiate the response. When T cell or macrophage function is compromised the entire complement of cell mediated immunity is affected. This leads to a profoundly immunocompromised state in the host. Of course, in infection this can be lethal, but in allograft rejection it can be life saving. Finally, DTH must be viewed not as a host response in and of itself but rather one component of an interrelated coordinated host response to disease.

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